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The protective effects of *Lactobacillus* SNK-6 on growth, organ health, and intestinal function in geese exposed to low concentration Aflatoxin B1

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ABSTRACT Aflatoxin B1 (**AFB1**) is a prevalent mycotoxin present in feed ingredients. In this study, we investigated the effects of *Lactobacillus salivarius* (***L. salivarius***) on the Landes geese exposed to AFB1. The 300 one-day-old Landes geese were randomly divided into five groups: The control group received a basic diet, while the other groups were fed a basic diet supplemented with 10 μg/kg AFB1, 10 μg/kg AFB1+ 4*10^8 cfu/g L. salivarius, 50 μg/kg AFB1, and 50 μg/kg AFB1 + 4*10^8 cfu/g *L. salivarius* for 63 d. Results showed that high level AFB1 exposure significantly decreased final BW and ADG, increased feed/gain ratio (F/G) and liver index ($P < 0.05$). *L. salivarius* improved levels of IL-1, IL-6, and IL-12 under low level of AFB1 exposure ($P < 0.05$), along with similar trends observed in serum IgA, IgG, IgM, T3, T4, TNF-α, and EDT ($P < 0.05$). AFB1 exposure reduced jejunum villus high and villus high/crypt depth ratio, and suppressed expression of *ZO-1, Occludin*, and *Claudin-1* mRNA, and significant improved with *L. salivarius* supplementation under low level AFB1 exposure ($P < 0.05$). AFB1 significantly increased expression levels of *TLR3* and *NF-kB1*, with supplementation of *L. salivarius* showing significant improvement under low AFB1 exposure ($P < 0.05$). Cecal microbiota sequencing revealed that under low level AFB1 exposure, supplementation with *L. salivarius* increased the abundance of Bacteroidetes and *Lactococcus*. In summary, supplementation with 4*10^8 cfu/g *L. salivarius* under 10 μg/kg AFB1 exposure improved growth performance and immune capacity, enhanced jejunum morphology, reduced liver inflammation, altered the cecal microbial structure, and positively affected the growth and development of geese.

Key words: goose, growth performance, liver, intestinal, cecum microbiota

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INTRODUCTION

Mycotoxin contamination in livestock feed presents a significant concern in livestock breeding, with a considerable amount of grains being contaminated by mycotoxins annually. Among these contaminants, aflatoxin contamination, especially from Aflatoxin B1, is of significant concern in poultry feed (Bryden, 2012; Schatzmayr and Streit, 2013). Aflatoxins, produced by *Aspergillus flavus* and *Aspergillus parasiticus*, are pivotal fungal toxins commonly found in animal feeds such as corn, peanuts, and rice (Awuchi et al., 2020). The main types of aflatoxins include B1, B2, G1, G2, M1, and M2, with Aflatoxin B1 being the most toxic variant (Huang et al., 2010). The adverse effects of AFB1 on humans have been well-established, including hepatotoxicity, respiratory toxicity, immunotoxicity, and reproductive toxicity, leading to its classification as a Group 1 carcinogen by the International Agency for Research on Cancer (Frangiamone et al., 2022). In addition to its severe health implications, Aflatoxin B1 significantly impacts poultry production, compromising their digestive and immune functions, resulting in reduced growth performance, reproductive performance, and immune dysfunction, as well as organ damage (Fouad et al., 2019). A addition of 60 μg/kg of AFB1 to their diet significantly impairs the growth performance of broilers at 42 d and increase the F/G (Zou et al., 2023). Similarly, adding 2 mg/kg of AFB1 in broiler diets can compromise their immune system, suppressing antibody production (Solis-Cruz et al., 2019). High levels of AFB1 have been shown to reduce the growth performance of 42-day-old

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broilers, elevate the activities of serum alanine aminotransferase (**ALT**) and aspartate aminotransferase (**AST**), damage liver function, induce oxidative stress, and trigger hepatocyte apoptosis (Guo et al., 2022; Sang et al., 2023). Moreover, Aflatoxin B1 exposure has adverse effects on poultry reproductive health. Song et al. found that feeding AFB1-contaminated feed to poultry for 4 wk resulted in decreased hatchability, fertility rate, fertilized egg hatchability, and increased embryo mortality trend (Song et al., 2023). Ducks are also vulnerable to AFB1, as evidenced by Shi et al., who impaired growth performance in day-old ducklings following administration of 40 μg/kg of Aflatoxin B1 on the seventh day (Zhang et al., 2023). Adding 60 μg/kg of AFB1 to the diet also damaged the mitochondrial function of the duck's liver, causing oxidative damage to the liver mitochondria (Liu et al., 2021). Another experiment confirmed that AFB1 contamination promotes duck hepatocyte apoptosis, reduces protease activity, and ultimately causes liver function damage (Liao et al., 2014). Moreover, AFB1 poisoning disrupts the intestinal absorption barrier in poultry, leading to poor absorption of essential trace nutrients (Yunus et al., 2011).

Currently, the primary methods for aflatoxin detoxification encompass physical and chemical approaches. For instance, the addition of aflatoxin binders in chicken diets, such as hydrated sodium calcium aluminosilicate, has been shown to reduce the AFB1 accumulation in the liver, increase the AFB1 excretion, and alleviate the relative liver weight (Liu et al., 2018). Certain fungi, Bacillus and *Lactobacillus* can effectively remove aflatoxins from feed (Xu et al., 2017; Yasmeen et al., 2021). *Lactobacillus plantarum,* for instance, enhances glutathione binding activity by inhibiting the expressions of CYP1A2 and CYP3A4, thereby inducing a detoxifying effect (Huang et al., 2017). *Escherichia coli* from chicken cecum effectively eliminates AFB1, with a reduction of up to 93.7% observed after 72h of aerobic culture (Wang et al., 2019). *Lactobacillus* SNK-6, a native strain from Xinyang black-boned laying hens discovered by Huaxiang Yan of the Shanghai Academy of Agricultural Sciences, has been found to improve the immune performance of laying hens and demonstrate significant effect in preventing pigeon diarrhea (Liu et al., 2022; Fan et al., 2024).

Poultry species exhibit variations in metabolic enzyme activities, detoxification mechanisms, and DNA repair capabilities, leading to differences in their sensitivity to AFB1 (Dohnal and Kuča, 2014). However, limited research has explored the impact of AFB1 on geese, and the tolerance of Landes geese, specifically bred for liver production, to AFB1 remains unclear. This experiment aims to study the effects of high and low levels of AFB1 exposure on the growth performance, liver, and intestines of Landes geese, along with the potential intervention outcomes with *L. salivarius* SNK-6. The objective is to explore microbial interventions as a solution to mitigate the adverse effects of AFB1 exposure on geese.

MATERIALS AND METHODS

Materials, Experimental Design, and Management

AFB1 (purity≥98%) was obtained from Pribolab Biological Technical Company, Qingdao, China. *L. salivarius* strain SNK-6 was provided by the Institute of Animal Science and Veterinary Medicine, Shanghai Academy of Agricultural Sciences. The bacterial strain was processed into bacterial powder by Jiangsu Weikang Biotechnology Co., Ltd. Yancheng, China, and stored at −80°C, with a live bacterial count of 2.0×10^{11} cfu/g.

Landes geese were acquired from Ma'anshan Xiangtian Ge Poultry, Nanjing, China. Three hundred 1-day-old Landes geese were randomly allocated into 5 groups, with 6 replicates per group and 10 geese per replicate. The control group received a basic diet (Con group), while the other groups were fed a basic diet supplemented with 10 μg/kg AFB1 (L group), 10 μg/kg AFB1+ $4*10^8$ cfu/g L. salivarius/per bird (LL group), 50 μg/kg AFB1 (H group), and 50 μg/kg AFB1 + $4*10^8$ cfu/g *L. salivarius* (HL group). The AFB1 were first uniformly mixed with a small quantity of feed, Then thoroughly blended with the main batch of feed to ensure even distribution. For *L. salivarius*, it was weighed and manually added to the feed of bird in each replicate before feeding. The diets were formulated according to the National Research Council; Subcommittee on Poultry Nutrition, 1994 standards and adjusted according to the nutritional needs of geese at different stages, ensuring consistent main nutritional levels across all groups (Supplementary Table 1). The pre-trial period lasted for 1 wk, followed by an 8-wk trial period. The feeding trial was conducted at the Zhuanghang Experimental Station Goose Farm of the Shanghai Academy of Agricultural Sciences, Shanghai, China. The geese were floor-raised on both sides of the same shed, positioned 1m above the ground level. Each cage, measuring 200cm*200cm*50cm, each cage is separated by netting to prevent geese from flying into other cages, with ad libitum access to feed and water. Manual feeding was performed twice daily (08:00 and 14:00), during the initial brooding period from d 1 to d 28, a warm-air heater was used to maintain the temperature within the goose house at a consistent 28-33℃ to ensure optimal growth conditions for the goslings. From d 28 to d 63, a total of 16 hours of natural and artificial light provided. Natural ventilation, alongside routine management practices and vaccination, adhered to the Landes geese breeding management requirements.

Growth Performance

The residual feed from the previous day was removed and weighed each morning before feeding, with daily feed intake calculated for each replicate. At the end of the experiment, at 08:00 (after an 8-h fasting period), the weight of each goose was recorded. The ADG, ADFI, and feed/gain ratio (F/G) were calculated from

the total feed intake and the total weight of the geese in each replicate.

Serum Indices

At the termination of the experiment, blood samples were collected from the wing vein of one goose from each replicate, chosen based on proximity to the average weight of that replicate. The samples were centrifuged at 4,000 r/min for 10 min to separate the serum using a Sorvall ST 40 centrifuge (Thermo Fisher Scientific, MA), and were then stored at −20°C for further analysis. Serum levels of ALT, ALP, GGT, AST, GLU, BUN, TC, TG, HDL-C, LDL-C were determined using a BS-200 auto biochemistry analyzer (Mindray, Shenzhen, China). Serum levels of IL-1, IL-6, IL-10, IL-12, IgA, IgG, IgM, T3, T4, TNF-α, DAO, and EDT were measured using an Infinite F50 plate reader (Tecan, Switzerland). All reagent kits were purchased from Shanghai Renjie Biotechnology Co., Ltd, Shanghai, China.

Organ Indices

Geese were euthanized via cervical dislocation, and organs including the heart, liver, gizzard, glandular stomach, thymus, intestines, kidneys, bursa of Fabricius, and spleen were removed and weighed. The immune organ index (immune organ weight, g/animal live weight, kg) was calculated, and the relative lengths of the duodenum, jejunum, and ileum were determined (relative length = intestinal length, cm/total intestinal length, cm). All procedures were approved by the Animal Care and Ethics Committee of the Shanghai Academy of Agricultural Sciences, and efforts were made to minimize animal suffering during sampling.

Intestinal and Liver Histology

Samples from the jejunum and ileum were collected and mixed with 4% paraformaldehyde for histological analysis. The intestinal tissues were then fixed, embedded in paraffin, stained with hematoxylin and eosin (**HE**), and sectioned. An Olympus BX-41TF microscope (Olympus Corporation, Tokyo, Japan) was used to measure the villus height (**VH**) and crypt depth (**CD**) of five morphologically normal, straight villi per section, and the ratio of VH/CD was calculated. The average measurement from five villi was recorded for each parameter.

Liver tissues were fixed overnight in 10% formalin solution, embedded in paraffin, and sectioned at 5μm thickness using a Leica rotary microtome (Leica Biosystems, Germany). HE staining was performed on the sections, and pathological changes in the liver were assessed under a Leica Aperio CS2 slide scanner (Leica Biosystems, Wetzlar, Germany).

Real-Time Quantitative PCR

RNA was extracted from the liver and jejunum using Trizol reagent (Invitrogen, Carlsbad, CA), followed by cDNA synthesis utilizing a reverse transcription kit (TransGen Biotech, Beijing, China). The concentration and purity of the resulting cDNA were determined using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, MA). The expression levels of target genes, including *ZO-1, Occludin, Claudin-1, IL-6, IL-10, TLR3, MyD88, NF-kB1, iNOS*, and *β-actin* (as an internal control), were quantified using the TransGen TB Green Premix (TransGen Biotech, Beijing, China) on a QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific, MA). Primer sequences are listed in Supplementary Table 2. Data analysis was performed using the $2^{-\Delta\Delta CT}$ method for relative quantification.

Cecum Microbiota

High-throughput sequencing was employed to analyze the microbial abundance in the cecal contents, facilitated by Personal Technology Co., Ltd., Shanghai, China. DNA extraction from the cecal contents, and sequencing of the V3-V4 regions of the microbial DNA were performed on an Illumina platform using paired-end sequencing. Primer sequences for amplification were F: ACTCCTACGGGAGGCAGC; R: GGACTACHVGGGTWTCTAAT. Subsequent data processing utilized the DADA2 pipeline for primer removal, quality filtering, denoising, merging, and chimera removal. The analysis was performed using QIIME2, with further steps including primer trimming using qiime cutadapt trim-paired and quality control using qiime dada2 denoise-paired. Data visualization and analysis were conducted on the GenesCloud platform (https://www.genescloud.cn/).

Data Analysis

Experimental data were preliminarily processed using Excel 2019 and subsequently analyzed with SPSS software (version 26.0, SPSS Inc., Chicago, IL) using one-way ANOVA. Differences between groups were assessed using Tukey's multiple comparison test, with $P < 0.05$ considered statistically significant. Results are presented as mean ± standard error of the mean (**SEM**).

RESULTS

Growth Performance

Table 1 presents data on initial body weight and average daily feed intake (ADFI), which remained consistent across all groups without notable disparities. Neither the L nor LL groups displayed significant deviations from the Con group in FBW, whereas the H and HL groups displayed a significant reduction in FBW compared to the Con and LL groups ($P < 0.05$). A similar trend was observed in ADG, where the L and LL groups mirrored the Con group's performance closely, unlike the H and HL groups, which showed significantly lower ADG compared to both the Con and LL groups

Table 1. Effects of *L. salivary* SNK-6 on growth performance of geese exposed to AFB1.

Items[1]	Groups[2]					SEM	*P*-value
	Con	L	LL	H	HL		
Inital BW, g	120.10	122.00	119.37	121.07	120.13	0.56	0.628
Final BW, g	4850.63[a]	4672.80[ab]	4779.80[a]	4422.93[b]	4492.90[b]	40.63	0.002
ADG, g	75.08[a]	72.23[abc]	73.97[ab]	68.28[c]	70.20[bc]	0.65	0.005
ADFI, g/d	312.12	311.80	315.29	306.08	317.51	3.74	0.917
F/G	4.16[c]	4.32[bc]	4.26[bc]	4.48[ab]	4.58[a]	0.04	0.009

[1]BW: body weight; ADG: average daily gain; ADFI: average daily feed intake; F/G: feed/gain ratio.
[2]Con = control group; L = 10 μg/kg AFB1 added in the diet; LL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet; H = 50 μg/kg AFB1 added in the diet; HL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet.
[a,b,c]Different letters superscripts mean significant differences ($P < 0.05$).

($P < 0.05$). In terms of the F/G, the L and LL groups aligned closely with the Con group, whereas the H and HL groups experienced a significant increase in F/G compared to the Con and LL groups, with the HL group exhibiting highest F/G ($P < 0.05$).

Organ Indices

Table 2 showed the variations in organ indices across different groups. To preliminarily assess the differences in immune performance among the groups, we measured the immune organ indices (bursa of Fabricius, thymus, spleen) and found no significant differences. In terms of liver and kidney indices, the L and LL groups did not differ significantly from the Con group, while the HL and H groups exhibited significant increases ($P < 0.05$). The relative lengths of the duodenum, jejunum, and ileum showed no differences among the groups. However, the intestinal index was highest in the H group and lowest in the Con group ($P < 0.05$).

Serum Indices

The impact of *L. salivarius* SNK-6 on the serum levels of geese treated with different levels of AFB1 is shown in Table 3. Regarding liver damage indicators, the ALP levels in the H and HL groups were significantly higher than those in the Con group ($P < 0.05$), with no significant difference from the L and LL groups. Moreover, ALT levels in the H and HL groups were significantly higher compared to the Con group as well as the L and LL groups ($P < 0.05$), while no significant differences were observed among the Con group, L group, and LL group. There were no discernible differences among the groups in terms of GGT, AST, GLU, BUN, TC, TG, HDL-C, LDL-C.

Serum Immune Indices

To further investigate the differences in serum immune levels among the groups, we measured the levels of IL-1, IL-6, IL-10, IL-12, IgA, IgG, IgM, T3, T4, TNF-α, DAO, and EDT (Table 4). Compared to the Con group, serum levels of IL-1 and IL-6 were significantly increased in the L, HL, and H groups ($P < 0.05$), with no significant difference observed in the LL group. Concerning IL-10 and IL-12 levels, the Con and LL groups had higher levels, yet no significant differences were observed among the groups. IgA, IgG, and IgM levels were lowest in the H and HL groups, significantly lower than those in the Con group ($P < 0.05$). Levels of IgA, IgG, and IgM in the Con group showed no difference from the LL group. Trends for T3 and T4 were consistent, with the Con and LL groups displaying significantly higher levels than the H and HL groups ($P < 0.05$), with no difference observed between the LL and Con groups. Serum EDT

Table 2. Effects of *L. salivary* SNK-6 on organ indexes of geese exposed to AFB1.

Items[1]	Groups[2]					SEM	*P*-value
	Con	L	LL	H	HL		
Heart index, g/kg	6.73	6.70	6.88	7.34	7.51	0.15	0.341
Liver index, g/kg	14.45[b]	15.42[b]	14.68[b]	17.96[a]	17.35[a]	0.045	0.026
Kidney index, g/kg	5.57[b]	5.43[b]	5.65[b]	6.88[a]	7.23[a]	0.22	0.010
Thymus index, g/kg	1.25	1.99	1.82	2.12	2.08	0.12	0.160
Fabricius index, g/kg	0.74	0.80	0.69	0.67	0.89	0.03	0.228
Spleen index, g/kg	0.59	0.47	0.49	0.74	0.67	0.04	0.093
Muscular stomach index, g/kg	37.79	39.38	35.90	40.38	42.97	0.93	0.156
Glandular stomach index, g/kg	2.92	3.10	2.77	3.15	3.32	0.08	0.255
Intestinal index, g/kg	33.44[c]	42.93[bc]	42.98[bc]	55.74[ab]	50.05[a]	2.17	0.008
Relative duodenum length, cm/cm	0.18	0.19	0.17	0.19	0.17	0.01	0.250
Relative jejunum length, cm/cm	0.37	0.37	0.35	0.39	0.35	0.01	0.153
Relative ileum length, cm/cm	0.45	0.44	0.48	0.41	0.48	0.01	0.059

[1]Organ indexes= organ weight, g/ animal live weight, kg.
[2]Con = control group; L = 10 μg/kg AFB1 added in the diet; LL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet; H = 50 μg/kg AFB1 added in the diet; HL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet.
[a,b,c]Different letters superscripts mean significant differences ($P < 0.05$).

Table 3. Effects of *L. salivary* SNK-6 on serum indices of geese exposed to AFB1.

Items[1]	Groups[2]					SEM	*P*-value
	Con	L	LL	H	HL		
ALP, IU/L	462.67[b]	526.75[ab]	494.83[ab]	567.50[a]	554.33[a]	11.78	0.025
ALT, IU/L	8.50[b]	12.92[a]	9.58[b]	13.33[a]	13.17[a]	0.54	0.004
GGT, IU/L	0.08	0.67	0.42	0.50	0.42	0.07	0.098
AST, IU/L	9.57	9.99	9.61	10.64	10.01	0.25	0.703
BUN, mmol/L	0.57	0.78	0.71	0.70	0.73	0.03	0.192
GLU, mmol/L	10.82	8.75	9.75	7.41	8.40	0.17	0.054
HDL-C, mmol/L	3.02[a]	2.74[bc]	2.86[ab]	2.52[c]	2.65[bc]	0.04	0.002
LDL-C, mmol/L	2.68	2.59	2.66	2.67	2.58	0.04	0.877
TC, mmol/L	4.83	4.80	4.95	4.92	4.78	0.06	0.854
TG, mmol/L	0.65	0.74	0.64	0.55	0.77	0.03	0.076
TP, g/L	41.68	39.88	39.31	40.39	39.84	0.30	0.112
ALB, g/L	15.42	14.97	15.15	15.37	15.28	0.10	0.680
GLOB, g/L	26.26	24.92	24.16	25.33	24.57	0.26	0.100

[1]ALT: alanine aminotransferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; GLU: Glucose; BUN: blood urea nitrogen; TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TP: total protein; ALB: albumin; GLOB: globulin.

[2]Con = control group; L = 10 μg/kg AFB1 added in the diet; LL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet; H = 50 μg/kg AFB1 added in the diet; HL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet.

[a,b,c]Different letters superscripts mean significant differences ($P < 0.05$).

Table 4. Effects of *L. salivary* SNK-6 on serum immue indices of geese exposed to AFB1.

Items	Groups					SEM	*P*-value
	Con	L	LL	H	HL		
IL-1, pg/mL	122.69[c]	172.39[b]	129.69[c]	193.57[a]	173.45[a]	5.55	<0.001
IL-6, pg/mL	78.99[c]	100.21[ab]	79.76[c]	104.15[a]	94.12[b]	2.29	<0.001
IL-10, pg/mL	257.94	245.82	265.21	229.24	241.71	5.76	0.166
IL-12, pg/mL	224.52	198.65	210.43	192.88	192.72	7.35	0.079
IgA, g/L	4.74[a]	3.92[bc]	4.30[a]	3.70[c]	3.69[c]	0.10	<0.001
IgG, g/L	13.01[a]	10.94[b]	12.14[a]	9.02[c]	10.37[b]	0.31	<0.001
IgM, g/L	1.51[a]	1.35[b]	1.49[a]	1.20[c]	1.25[c]	0.03	<0.001
T3, mmol/L	43.53[a]	35.70[b]	44.27[a]	40.11[ab]	39.50[ab]	0.93	0.015
T4, mmol/L	6.75[a]	5.49[b]	6.86[a]	5.71[b]	5.56[b]	0.14	<0.001
TNF-α, pg/mL	214.71[c]	299.70[a]	253.44[b]	306.03[a]	291.58[a]	7.74	<0.001
DAO, mmol/L	20.00[a]	15.50[bc]	18.72[a]	14.19[c]	18.04[ab]	0.55	0.001
EDT, mmol/L	388.61[a]	501.60[b]	432.25[a]	595.01[c]	498.09[b]	14.66	<0.001

[1]IL-1: Interleukin-1; IL-6: Interleukin-6; IL-10: Interleukin-10; IL-12: Interleukin-12; IgA: Immunoglobulin A; IgG: Immunoglobulin G; IgM: Immunoglobulin M; T3: triiodothyronine; T4: thyroxine; TNF-α: tumor necrosis factor-alpha; DAO: diamine oxidase; EDT: ethylenediaminetetraacetic acid.

[2]Con = control group; L = 10 μg/kg AFB1 added in the diet; LL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet; H = 50 μg/kg AFB1 added in the diet; HL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet.

[a,b,c]Different letters superscripts mean significant differences ($P < 0.05$).

levels were higher in the L and H groups, showing a decreasing trend in the LL and HL groups, and lowest in the Con group ($P < 0.05$). In contrast, serum DAO levels were inversely related to EDT levels, with the highest levels observed in the Con and LL groups, significantly higher than those in the L and H groups ($P < 0.05$).

Intestinal Morphology

HE sections of the jejunum and ileum are shown in Figure 1. The Con group exhibited tightly packed intestinal villi with intact epithelial morphology. In contrast, the L, H, and HL groups displayed swollen and ruptured villi, while the LL group showed relative improvement. No significant differences in CD were observed among the groups (Figures 1B and 1E). In the jejunum, the Con group and LL showed no significant difference, being significantly higher than the L, H, and HL groups in VH ($P < 0.05$) (Figure 1A). Additionally, the LL group had the highest VH/CD ratio, surpassing the L, H, and HL groups (Figure 1C). In the ileum, the VH of the L group showed no difference compared to the Con and LL groups but was significantly higher than that of the H and HL groups ($P < 0.05$) (Figure 1D).

Liver Histology

Liver tissues from the control group showed intact and clear lobular structures, neatly arranged hepatocyte plates, and abundant cytoplasm, with portal areas clearly demarcated. In contrast, liver tissues from the AFB1-treated groups displayed blurred lobular structures, disorganized liver cords, with some areas showing the disappearance of cord-like structures. Hepatocytes appeared enlarged and rounded, with the H group showing ballooning degeneration, nuclear migration towards the cell membrane, disruption of the intact membrane structure, and unclear boundaries. Additionally, some

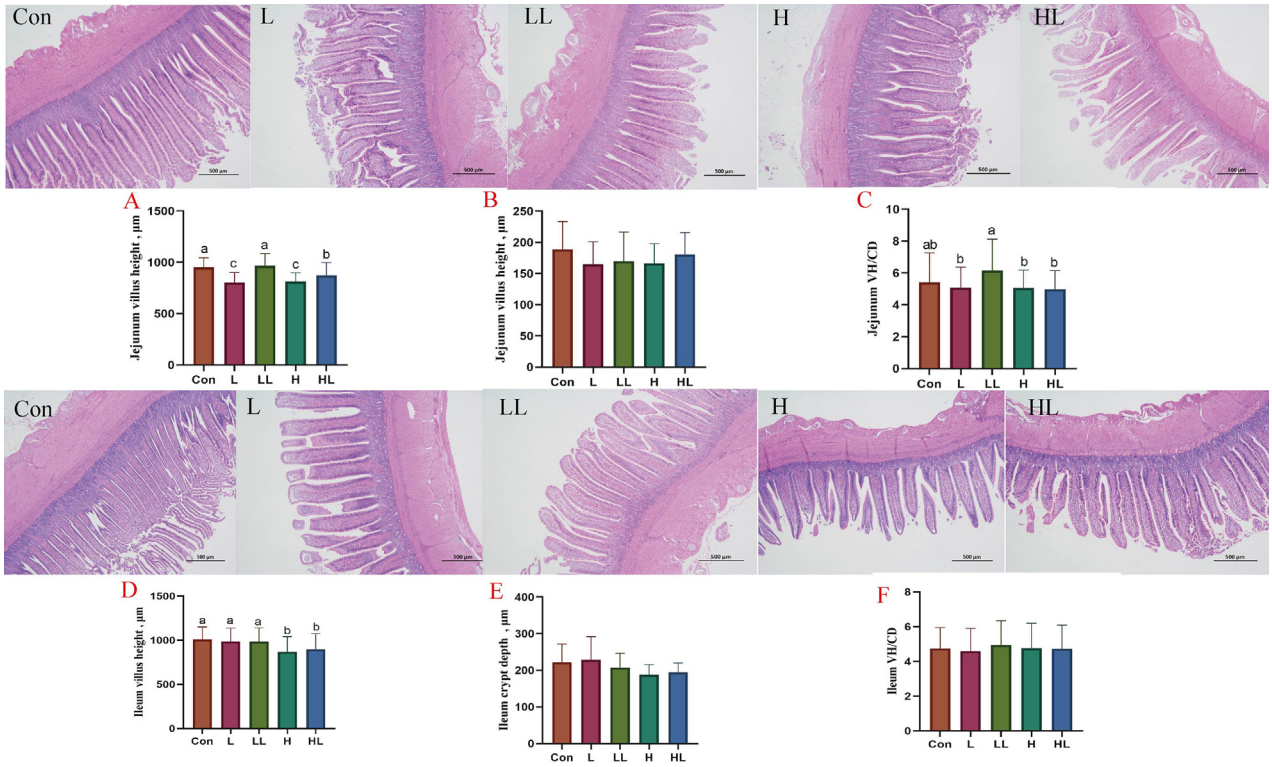

Figure 1. Effects of *L. salivary* SNK-6 on Intestinal morphology of geese exposed to AFB1. VH (Villus height), CD (crypt depth). Con = control group; L = 10 μg/kg AFB1 added in the diet; LL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet; H = 50 μg/kg AFB1 added in the diet; HL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet.

cells exhibited diffuse fatty vacuolar degeneration, with portal areas showing signs of inflammatory cell infiltration. Following intervention with *Lactobacillus* SNK-6, the LL group's liver morphology normalized compared to the control group, showing improvement over the L group. Notably, no significant pathological changes such as hepatocyte degeneration, swelling, or inflammatory infiltration were observed in the LL group (Figure 2).

Real-Time Quantitative PCR

As shown in Table 5, exposure to AFB1 significantly altered the intestinal permeability in the jejunum of the H and HL groups, resulting in significant reductions in *ZO-1, Occludin*, and *Claudin-1* ($P < 0.05$), while supplementation with the *L. salivarius* SNK-6 showed improvement under low-level AFB1 exposure, with no significant difference from the Con group. The differences in *IL-6* and *IL-10* levels among the groups were not significant in the spleen. Regarding pro-inflammatory factors in the liver, *TLR3, MyD88, iNOS*, and *NF-kB1* showed an increasing trend, but supplementation with *L. salivarius* SNK-6 improved the expression levels of pro-inflammatory factors, especially under low levels of AFB1 exposure. Specifically, the expression levels of *TLR3* and *NF-kB1* in the H group were significantly higher than in the Con and LL groups ($P < 0.05$), while the L group showed an upward trend but no significant difference from the control and LL groups. However, there were no significant differences in *MyD88* and *iNOS* expression among the groups. These results suggest that *L. salivarius* SNK-6 can improve intestinal permeability under AFB1 exposure and somewhat mitigate the liver inflammation levels.

Table 5. Effects of *L. salivary* SNK-6 on genes expression of geese exposed to AFB1.

Items	Groups					SEM	*P*-value
	Con	L	LL	H	HL		
ZO1	1.00^{ab}	0.85^{b}	1.15^{a}	0.54^{c}	0.75^{bc}	0.05	<0.001
Occludin	1.00^{a}	0.76^{b}	0.93^{ab}	0.44^{c}	0.45^{c}	0.05	<0.001
Claudin1	1.00^{a}	0.80^{bc}	0.89^{ab}	0.65^{cd}	0.51^{d}	0.04	<0.001
IL6	1.00	1.57	1.21	1.92	1.40	0.13	0.238
IL10	1.00	1.54	1.48	1.79	2.09	0.17	0.341
TLR3	1.00^{b}	1.39^{ab}	1.23^{b}	2.29^{a}	1.76^{ab}	0.1	0.047
myd88	1.00	1.62	1.24	2.38	2.32	0.21	0.138
NFκB1	1.00^{b}	1.38^{ab}	1.40^{ab}	2.26^{a}	1.96^{ab}	0.17	0.041
iNOS	1.00	1.39	1.07	1.97	1.70	0.14	0.160

[1]*ZO-1*: Zonula Occludens-1; *IL-6*: Interleukin-6; *IL-10*: Interleukin-10; *TLR3*, Toll-like Receptor 3; *Myd88*: myeloid differentiation primary response 88; *NF-Kb*: nuclear factor kappa-light-chain-enhancer of activated B cells; *iNOS*: inducible nitric oxide synthase; *β-actin*: Beta-actin.

[2]Con = control group; L = 10 μg/kg AFB1 added in the diet; LL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet; H = 50 μg/kg AFB1 added in the diet; HL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet.

[a,b,c]Different letters superscripts mean significant differences ($P < 0.05$).

Cecum Microbiota Alpha Diversity

To comprehensively evaluate the alpha diversity of microbial community, richness was characterized by

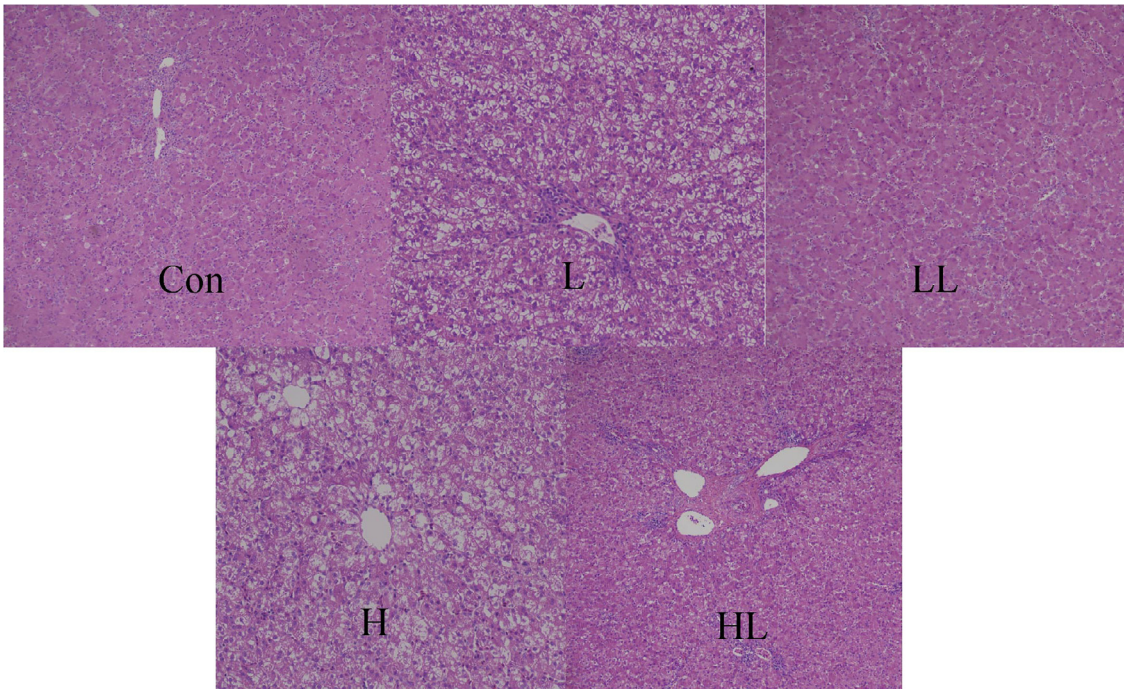

Figure 2. Effects of *L. salivary* SNK-6 on liver morphology of geese exposed to AFB1. Con = control group; L = 10 μg/kg AFB1 added in the diet; LL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet; H = 50 μg/kg AFB1 added in the diet; HL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet.

Chao1 and Observed species indices, diversity by Shannon and Simpson indices, phylogenetic diversity by Faith's PD index, and evenness by Pielou's evenness index (Figure 3). In terms of gut microbiota richness, the trends of Chao1 and Observed species were consistent across groups, with the highest observed in the control group. However, there was no difference in Chao1 between the control and LL groups, which were significantly higher than the H and L groups ($P < 0.05$). Regarding diversity, the Shannon and Simpson indices were also lower in the H and L groups, showing significant differences from the control group, while the LL group showed an increase ($P < 0.05$). Consistent trends were observed in Pielou's evenness and Faith's PD indices ($P < 0.05$).

Cecum Microbiota Beta Diversity

Differences in bacterial community composition among the groups were assessed using Principal Coordinate Analysis (**PCoA**) based on the Bray-Curtis distance algorithm (Figure 4A). The PCoA results indicated that the PCO1 accounted for 21.3% and PCO2 for 13.5% of the variation, with the control group samples distinctly clustering, and a larger separation observed within the H group samples. Furthermore, NMDS analysis, a key indicator of sample differences, yielded a stress value less than 0.2 (stress=0.185), indicating significant differences between samples. Interestingly, some overlap was observed between the LL and HL group samples, suggesting smaller differences between these groups (Figure 4B).

Cecum Microbiota Composition Analysis

The analysis of the top 10 categories at the phylum and genus levels across all samples is illustrated in Figure 5. At the phylum level, Firmicutes showed the highest abundance (56.42−78.25%), followed by Actinobacteria (3.92−19.44%), Proteobacteria (7.96−23.42%), and Bacteroidetes (0.02−26.70%) as shown in Figure 5A. At the genus level (Figure 5B), the highest abundance was observed for *Enterococcaceae_ Enterococcus* (0.87−14.07%), with subsequent prevalences for *Shigella* (0.14−20.51%), *Turicibacter* (0.17−12.14%), and *Bacteroidaceae_ Bacteroides* (0.00−14.57%).

To further compare the differences in species composition among the samples, visualize the distribution trends of species abundance, and identify marker species among different groups, we utilized the abundance data of the top 20 genera to construct LEfSe diagrams (Figure 5C). At the genus level, the Con group harbored the most biomarkers, including *Bacteroides, Megamonas, Desulfovibrio, Barnesiella, Faecalibacterium, Oscillospira, Selenomonas, Alistipes, Anaerobiospirillum*. The LL group featured *Streptococcus, Lactococcus*, and *Agrobacterium*. Biomarkers for the HL group included *Aerococcus, Acinetobacter, Moraxella*, and *Kurthia*, while the H group was characterized by *Butyricicoccus*. The biomarkers for the L group comprised *Shigella, Caulobacter*, and *Dietzia*.

DISCUSSION

Feeding is fundamental to the metabolic processes of animals, with intake easily influenced by factors such as

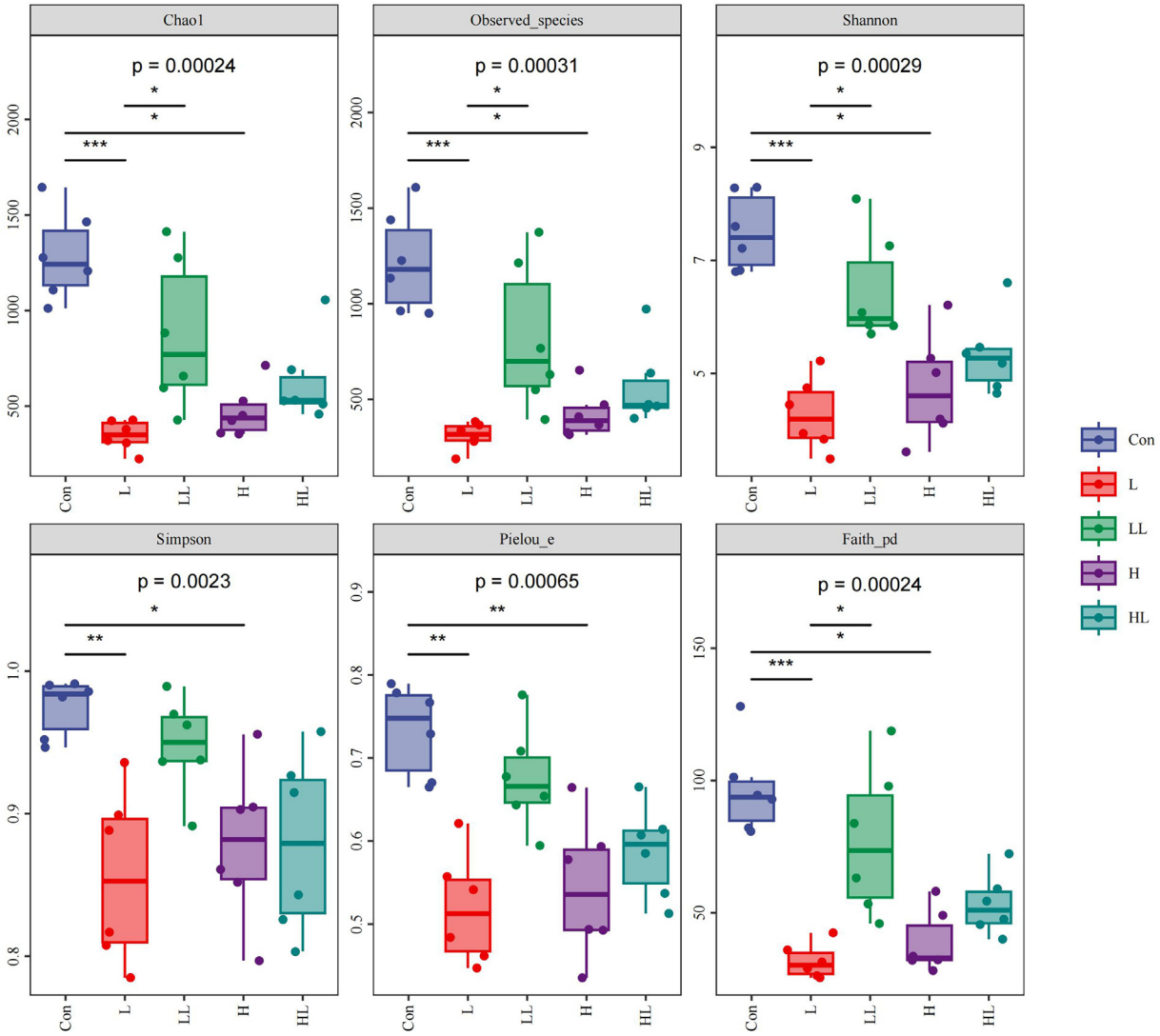

Figure 3. Alpha diversity index. Con = control group; L = 10 μg/kg AFB1 added in the diet; LL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet; H = 50 μg/kg AFB1 added in the diet; HL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet.

feed quality, environmental conditions, and the health status of the animal. External stimuli, in particular, can have a pronounced impact on feed intake, often leading to reductions. AFB1 serves as one such stimulus; when ingested, it stresses the animal's body, thereby affecting its feed intake (Alvarado et al., 2017). The earliest and most evident symptoms of mycotoxin contamination in animals include growth stunting and reduced feed intake, resulting from a decline in feed quality, digestive enzyme levels, and immunocompetence, among other factors (Malekinezhad et al., 2021; Tolosa et al., 2021). In this experiment, we observed no difference in ADFI among groups. This lack of variation could be attributed due to the dosage, as the harm caused by AFB1 to broilers and the dose of AFB1 are correlated. At concentrations of AFB1 in feed lower than 0.1 mg/kg, broilers typically exhibit slowed growth, reduced digestibility, and imbalances in intestinal microbial composition (Chang et al., 2020). Once the dose reaches 0.4 mg/kg, a significant decline in broiler growth performance, microscopic organ damage, and lowered organ indices are observed (Zuo et al., 2013). At toxin levels above 1.0 mg/kg, visceral organs like the liver and kidneys may suffer substantive damage or even result in death (Śliżewska et al., 2019). However, factors such as breed, nutritional components, and management complicate the relationship between AFB1 dosage and its toxic effects.

Recently, the use of plant-derived *Lactobacillus* and *Bacillus subtilis* as feed additives has significantly increased in the poultry industry. Studies have found that besides improving gut health in animals, these additives can also degrade AFB1. In laying hens, the combined use of 2 strains of *Bacillus subtilis* (ANSB060 and ANSB01G) reduced the bacterial load required for AFB1 reduction and delayed the appearance and concentration of AFB1 in eggs (Jia et al., 2016). With the application of *Lactobacillus plantarum* 299v, the weight gain of broilers suffering from aflatoxicosis recovered by 20-55% (Khanian et al., 2019). Supplementing broiler diets with *L. salivarius* at 10^7, 10^8, and 10^9 CFU/kg resulted in weight gains of 1.2, 2.9, and 2.9%, respectively, and a reduction in F/G by 1.3, 3.5, and 3.7%. In vitro experiments also showed that incubating *L.*

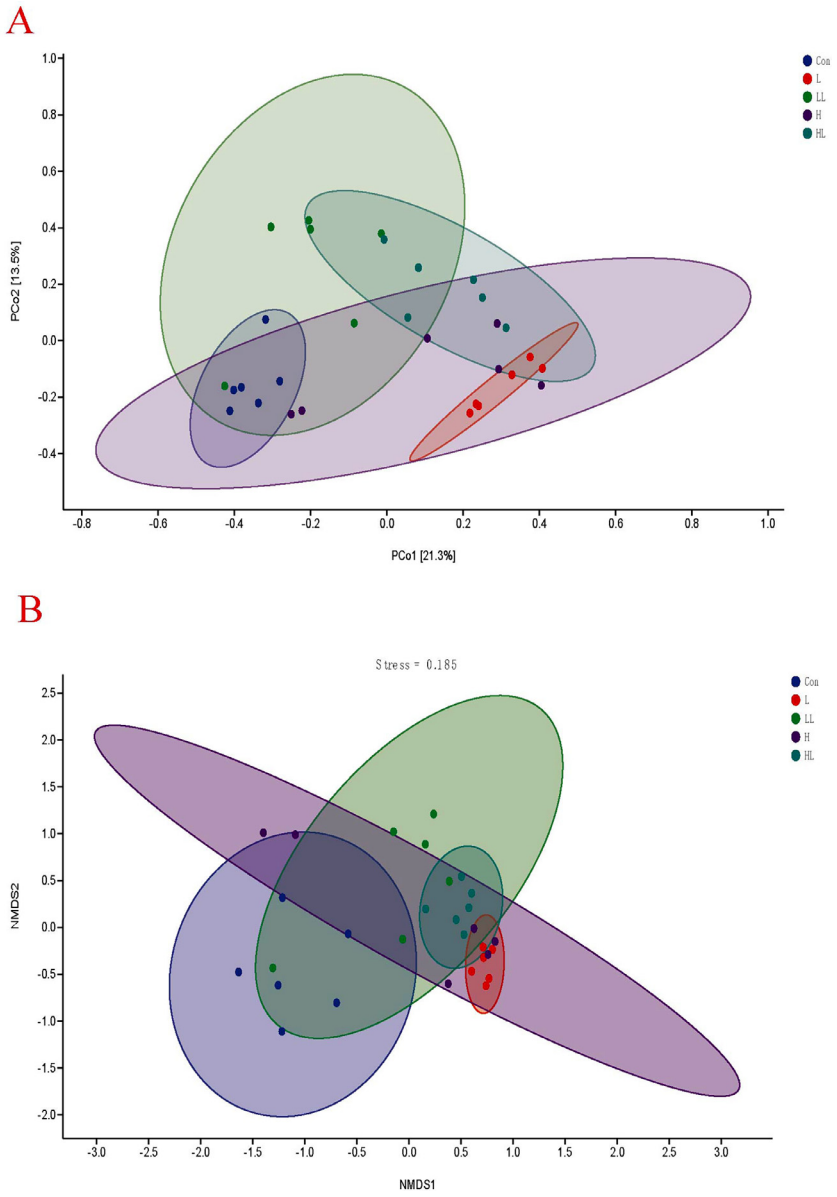

Figure 4. Beta diversity index. (A) Principal coordinate analysis (PCoA) plot of the bacterial community. (B) Nonmetric multidimensional scaling analysis (NMDS) plot of the bacterial community. Con = control group; L = 10 μg/kg AFB1 added in the diet; LL = 10 μg/kg AFB1 +4*10^8 cfu/g L. salivarius SNK-6 added in the diet; H = 50 μg/kg AFB1 added in the diet; HL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet.

salivarius with AFB1 for 12, 24, 48, and 72 h led to degradation rates of AFB1 at 46.9, 65.3, 86.7, and 91.5%, respectively (Chen and Wang, 2022). In this trial, we noticed that AFB1 hindered the growth of geese, regardless of whether they were treated with 10 μg or 50 μg/kg AFB1. However, when treated with 10 μg/kg AFB1 along with *L. salivarius* SNK6, the growth performance of geese almost matched that of the control group in terms of FBW, ADG, and F/G. Therefore, we believe that *L. salivarius* SNK6 can improve growth performance under AFB1 exposure and mitigate the impact of AFB1 contamination in feed.

The liver, as the largest digestive and metabolic organ in animals, plays a crucial role in the degradation, metabolism, and excretion of harmful substances (Li et al., 2015). It is considered the primary target organ for AFB1, with an increased liver weight being one of the evident symptoms. Chickens exposed to 2 mg/kg of AFB1 exhibited a 51.58% increase in relative liver weight and a lower liver color score (Shannon et al., 2017). Assessing changes in blood biochemical markers is important for determining the extent of liver damage in animals. ALP, an enzyme present in various tissues and organs, including the liver, bile ducts, bones, and intestines, mainly comes from bile duct epithelial cells in the liver. Its levels are closely linked to liver metabolism; when liver cells are damaged or destroyed, ALT is released into the bloodstream, causing higher ALT levels. Elevated ALT levels are often associated with liver diseases, such as hepatitis, cirrhosis, and liver cancer (Kalas et al., 2021).

Studies have shown that one of the significant hepatotoxic effects of AFB1 stems from its rapid absorption in the gastrointestinal tract, interaction with albumin and

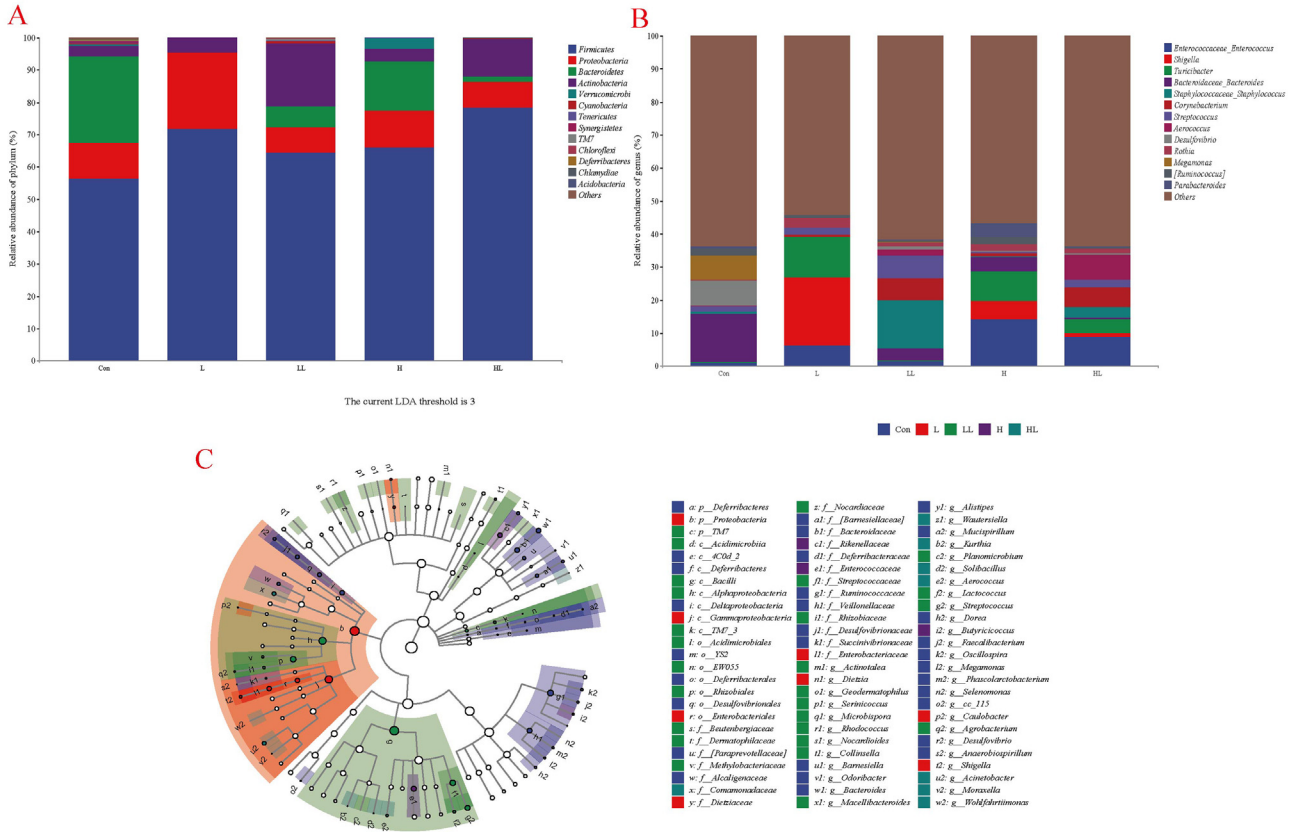

Figure 5. Structure of cecum microbiota. (A) Bar plots at the phylum level. (B) Bar plots at the genus level. (C) Linear discriminant analysis Effect Size (LEfSe) histogram showing the LDA scores (>3.0) computed for features at the gene level.Con = control group; L = 10 μg/kg AFB1 added in the diet; LL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet; H = 50 μg/kg AFB1 added in the diet; HL = 10 μg/kg AFB1+4*10^8 cfu/g L. salivarius SNK-6 added in the diet.

proteins, and retention in the liver. Liver microsomes convert AFB1 into unstable toxic metabolites, which then covalently bind to RNA, triggering significant biochemical reactions within the liver (Tolosa et al., 2021). This leads to lipid accumulation and pigment deposition in the liver, destroying liver cells, and affecting liver metabolism and immune function (Malekinezhad et al., 2021). In this study, we noticed significant changes in liver color and shape due to AFB1 exposure. When combined with ALP and ALT levels, it suggests severe liver damage and inflammation caused by AFB1. Toll-like receptors are responsible for recognizing pathogen-associated molecular patterns and damage-associated molecular patterns (DAMPs). The intake of AFB1 and the formation of its metabolites might be recognized by liver cells as DAMPs, thus activating *TLR3* (Wang et al., 2022), which in turn promotes downstream signal transduction, including the activation of *NF-κB*. AFB1 can also affect *NF-κB* activity by activating apoptosis-related signaling pathways such as p53, leading to cell death (Chen et al., 2021). This cell damage and death caused by AFB1 can further trigger an inflammatory response.

In this experiment, we also observed an increase in serum ALP and ALT levels and liver *TLR3, NF-κB1* expression under AFB1 exposure. However, with the supplementation of *L. salivarius* SNK6, there was a trend towards reduction. We believe this might be due to the strong degradative effect of *L. salivarius* on AFB1 (Chen and Wang, 2022), reducing its absorption and bioavailability, and its ability to secrete anti-inflammatory substances like short-chain fatty acids (Zhai et al., 2020), thereby reducing its damage to the liver.

The intestine, also recognized as a target organs for AFB1, experiences mucosal damage due to exposure, significantly increasing the body's vulnerability to external chemicals and pathogens (Ren et al., 2019). Tight junction proteins between intestinal epithelial cells are critical for intestinal barrier, and their decrease is common in chronic intestinal inflammatory diseases (Awad et al., 2017). To demonstrate intestinal barrier damage, the qPCR method is commonly used to measure the mRNA expression levels of tight junction proteins. Occludin and Claudin proteins are transmembrane proteins that connect with adjacent cells, acting as bridges to close the gaps between neighboring cells (Cummins, 2012). The ZO protein family serves as cytoplasmic scaffolding proteins, functioning as positioning pivots within the plasma membrane (Anderson et al., 1988). The levels of Occludin, Claudins, and ZO-1 proteins are often used as indicators of the integrity of the intestinal mucosal barrier. Studies have found that AFB1 can disrupt the tight junctions of the animal intestine. Administering 0.3 mg/kg of AFB1 daily to 4-wk-old mice and using immunofluorescence histochemical staining methods revealed changes in the distribution of Occludin,

Claudin-1, and ZO-1 tight junction proteins, disrupting their transport and localization (Li et al., 2023). In Caco-2 cells, a significant downregulation of *ZO-1* and *occludin* protein expression was observed in the toxin group (Gao et al., 2017). Interestingly, in this experiment, we found that exposure to 10 μg/kg of AFB1 alone resulted in the highest level of jejunal *claudin-1* expression. This aligns with findings in broilers exposed to 10-30mg/L of AFB1, suggesting a link to chronic rather than acute exposure. However, the expression trends for *ZO-1* and *occludin* were inconsistent, especially under 50 μg/kg AFB1 exposure (Zhang et al., 2022). Under the intervention of *L. salivarius* SNK-6, the expression of *ZO-1* and *occludin* significantly improved under 10μg/kg AFB1 exposure. Previous studies have shown that AFB1 damages basic intestinal functions, leading to microbial translocation. More bacterial translocation occurs when tight junction proteins like claudin, occludin, and ZO-1 are affected by AFB1 (Gratz et al., 2007). *L. salivarius* has been reported to limit translocation caused by exogenous toxins, and the secretion of anti-inflammatory substances like short-chain fatty acids by *L. salivarius* can improve, reducing oxidative damage and inflammation caused by translocation, effectively decreasing the intestinal permeability caused by AFB1 (Zhai et al., 2020). Through intestinal microbiome sequencing, we directly observed how AFB1 affected the gut microbiome. Both 10 μg/kg and 50 μg/kg AFB1 significantly reduced the richness and evenness of the geese's cecal microbiota. When examining species composition at the phylum level, the AFB1-treated groups had less abundant the Bacteroidetes phylum and more of the Proteobacteria phylum compared to the control group. The research results reported so far are not entirely consistent with the results obtained in this study. After ingestion of AFB1-contaminated feed, 16sRNA sequencing of the intestinal microbiota of *Litopenaeus vannamei* showed a decrease in microbial richness, an increase in the expression abundance of Proteobacteria bacteria, and a decrease in the expression abundance of Bacteroidetes (Wang et al., 2018). Mice or broilers ingesting AFB1 showed no significant difference in intestinal microbiota at the phylum level (Wang et al., 2016; Tavangar et al., 2021). At the genus level, results show that the abundance of the *Lactococcus* was a biomarker under supplementation with *L.salivarius* SNK-6 after exposure to 10 μg/kg. The *Lactococcus* has functions such as regulating the intestinal microbiota, enhancing immunity, protecting the gastric mucosa, and improving intestinal function. The abundance of *Lactobacillus* did not show a difference, and some studies have found that some *Lactobacillus* are consumed by AFB1, suggesting a competitive relationship between *L. salivarius* and AFB1 in the cecum, which in turn led to an increase in the abundance of the *Lactococcus*. Nonetheless, marker species like Barnesiella and Faecalibacterium in the control group were found to be at optimal abundance levels. Therefore, we believe that AFB1 can change the cecum microbiota depending on the dosage. Intervening with *L. salivarius* SNK6 intervention under low AFB1 exposure doses can somewhat enhance the richness and diversity of the cecal microbiota.

In summary, our findings indicate that while supplementation with $4*10^8$ CFU/g *L. salivarius* SNK-6 had positive effects on the immune response, liver and intestinal health, and cecal microbiota structure under 10 μg/kg AFB1 exposure. While *L. salivarius* shows potential as a dietary supplement, its effectiveness under conditions of AFB1 exposure requires further investigation. Future studies should explore the mechanisms underlying these interactions to better understand the potential of probiotic interventions in enhancing resilience to mycotoxin exposure.

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2024.103904.

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